Chapter 2

Buruli ulcer epidemiology and disease severity in Benin: data reported to the National Program, 2008-2019

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Manuscript in preparation

Abstract

Background

Data on the incidence and distribution of Buruli ulcer in Benin have been reported from some, but not all endemic foci in the country.

Methods

We analysed data on Buruli ulcer entered on the BU02 forms from all treatment centres in Benin, between 2008 and 2019.

Results

A consistent decline over time was noticed, from 897 patients in 2008 to 240 patients in 2019. Confirmation by PCR that started in 2010, gradually increased to 54.2% in 2019. The confirmation rate increased from 84.8 % in 2010 to 90.8% in 2019. The spatial distribution did not change markedly over time; PCR confirmation was similar in the four different referral centres, with one third of all cases being detected and reported from Zagnanado. Gender distribution was almost equal, median age was 17 (IQR 9-36) years. Almost half of the patients treated for BU during the study period had severe lesions (47.4%) with a predominance of ulcerative lesions (66.3%). A functional limitation at the start of treatment was observed in 25.4% of patients. Lesions were predominantly located on the lower limbs (62.8 %). The majority of patients (93.8%) received antimicrobial treatment. Category III lesions of BU (the severest form) increased from 43.6% in 2008 to 60% in 2019. This increasing trend was observed at all the treatment centres, except for Pobè where the category three lesions went down from 44.9% in 2008 to 31.9% in 2019.

Conclusion

Incidence of BU in Benin appears to have steadily declined; PCR confirmation rate has steadily increased; and antimicrobial treatment has been endorsed by all treatment centres. The increase in category III lesion incidence at detection suggests that a more active community surveillance is needed to improve BU control in Benin.

Introduction

Buruli ulcer (BU) or *Mycobacterium ulcerans* infection is one of the Neglected Tropical Diseases (1). BU usually starts as a non-ulcerative lesion - as a nodule, plaque or edema. These manifestations may eventually progress to massive skin ulcerations or bone infection when detected late or left untreated. Although BU is generally not fatal, people with BU might be left with cosmetic or functional deformity that can last a life time in the absence or a delay in effective treatment (1, 2). Standard laboratory techniques used to confirm BU are culture, histopathology, acid-fast bacilli microscopy, and PCR targeting a non-coding Insertion Segment (IS2404) that has multiple copies in each genome (1, 3). Formerly, surgery consisting of debridement with resection of a wide margin of apparently non-affected tissue was considered the only way to obtain cure. Today, although surgical practice persists in some hospitals, treatment consists of oral antibiotic therapy (clarithromycin and rifampicin combined) for eight weeks, combined with wound and lymphoedema management, and prevention of disability and rehabilitation (4-6).

To date, the disease has been reported in more than 30 countries, mainly in tropical regions with hot and humid climates, with the highest concentrations of patients in Côte d'Ivoire, Benin, Ghana, Cameroon, Democratic Republic of the Congo, Nigeria, and Australia (1). Globally, the number of BU patients is declining, but in some locales in Australia, Nigeria and Liberia, the incidence has increased (7-9). In Australia there has been a change in the epidemiology and the pathogenicity of BU. For example, in the state of Victoria, the number of reported patients has more than quadrupled, from 66 to 275 patients per year between 2013 and 2017. Moreover, the disease has spread to new geographic areas and the clinical manifestation have become more severe (7, 10-12).

The disease was diagnosed for the first time in Benin in 1977 (13) and the country is historically known as one of the most endemic countries for BU in West Africa. Like many others endemic countries (Ghana, Cameroon, Côte d'Ivoire), it is facing a decrease in the number of BU patients in recent years (1, 14, 15). A recent study on the evolution of epidemiological and clinical characteristics of BU from 2006 to 2017 revealed a decrease in prevalence of BU in the region with less severe lesions diagnosed (16). This observation was made in the district of Lalo, one of the endemic districts of Benin. However, there is a difference between endemic areas in terms of treatment strategy, health seeking behavior and community activities. Indeed, despite the introduction of antibiotic therapy in the treatment of BU, surgery remains a practice in some BU referral hospitals, with a large variation between hospitals (5). Fear of surgery has been associated with delay in seeking help in the official health care system (17, 18).

Community activities have been implemented in some endemic provinces such as active case finding, community awareness sessions and the decentralization of care for patients with BU (19-21). In all endemic provinces, there are community-based surveillance teams

that include village volunteers, teachers and community workers, supervised by health workers, but their work organization varies (19, 20, 22, 23). In the Zou province, a highly endemic area for BU with many patients presenting with severe lesions, patients are referred to the reference hospital, while in other endemic provinces patients are referred to the peripheral health center as part of the decentralization strategy (21). This may lead to a difference in the reduction of the number of BU patients as reported in Lalo.

This study aims to investigate the epidemiology of BU at a national level in order to understand the current distribution, the geographical patterns and the severity of the disease in Benin.

Materials and methods

Strategies implemented for BU control

Since its establishment in 2000, the BU National Control Program has implemented several disease control strategies inspired by the WHO guidelines. In terms of surveillance and screening, community-based surveillance teams were established for community activities such as active case finding. These teams refer patients to health systems if needed (19, 20, 22, 23). The teams are similar across different endemic areas. A form developed by the WHO (WHO BU02) is used as the data collection tool for surveillance purposes (23, 24). Data from all BU patients across Benin are collected using the WHO BU02 form and reported to the National Control Program. The National Reference Laboratory for Mycobacteria started to operate PCR targeting Insertion Segment 2404 (IS2404) for BU laboratory confirmation in 2010 (25, 26). However, the PCR test is expensive and requires technical expertise in terms of DNA extraction and equipment needed. To date, only the reference hospital of Pobè has the required logistics to carry out this PCR test on site. The other hospitals do not have the PCR on site, and they send samples to the National Reference Laboratory for Mycobacteria for diagnostic confirmation; results may take weeks to become available (27). In terms of treatment, antibiotic therapy was introduced in 2004, which made it possible to set up outpatient management of BU patients at peripheral health centers (21, 28, 29).

Study site

The study was carried out in Benin, West Africa. The BU control activities are organized by a National Control Program that in turn hinges on four BU Detection and Treatment Hospitals (CDTUB) distributed throughout the BU-endemic regions, all located in the south of the country (Figure 1). BU among patients with ulcers has not been detected in the northern part of Benin to date.

Figure 1 Location of different Buruli ulcer reference hospital in Benin

Study design and population

A retrospective, descriptive study was carried out over the period from 2008 to 2019. Data used in this study were obtained using the BU02 form from the WHO and they were retrieved from the database of the BU National Control Program. Data from patients treated for BU in all four BU's references hospitals in Benin (Allada, Lalo, Pobè, and Zagnanado) were included in the study.

Study parameters

To describe geographical distribution of BU, the following variables were collected: province, district, and the year of diagnosis.

To describe the socio-demographic, clinical and laboratory characteristics of the study population, the following variables were collected: age, sex, clinical features (ulcer, non-

ulcer), WHO category (category I, II or III), site of lesion (upper limb, lower limb, mixed, and others), functional limitations at admission, antibiotic treatment, laboratory diagnosis by PCR and PCR result. This data was collected from the WHO BU02 form.

In terms of severity, the WHO has classified BU into three categories. Category I lesions are single small lesions e.g. nodules, papules, plaques, and ulcers less than 5 cm in diameter, Category II lesions consist of non-ulcerative or ulcerative plaques, edematous forms, single large ulcerative lesion of 5–15 cm in cross-sectional diameter. Category III lesions are either at critical sites—notably, the face, breast and genitals; or disseminated and mixed forms including osteomyelitis, and extensive lesions of more than 15 cm (30).

Data analysis

Socio-demographic, clinical and laboratory data were collected and analyzed using SPSS version 26 (IBM). General descriptive is reported as median (IQR 25–75) for age, and frequency (%) for sex, ulcerative lesion, WHO category, and site of lesion, functional limitation at admission, PCR test performed, PCR result and antibiotics treatment. The differences in the severity (WHO category), ulcerative lesion (ulcer or not), functional limitation at admission, body site and treatment were compared between hospitals using Pearson chi-square, or Fischer exact test as appropriate.

The evolution of the number of BU patients over time is represented by a histogram showing the proportions for all treated patients and PCR confirmed patients. The evolution of lesions severity (WHO Category III lesions) over time is represented by curves showing the proportions for treated patients and within hospital.

Geographic data are illustrated using ARVIEW3.4 software. Map national distribution per district over a time intervals 5 years (2008 – 2013 – 2019) for all treated patients and Map national distribution per district in the years 2010 – 2013 – 2019 for PCR confirmed patients alone.

Ethics statement

Data in this study were used with the approval of the Ministry of Health of Benin, reference number 1953/MS/DC/SGM/DRFMT/SA. Patient information was anonymized by the assignment of identification numbers to protect the privacy of patients and the confidentiality of personal information.

Results

Number of BU patients

In total, 5055 patients were treated as BU from 2008 to 2019 in Benin. Out of these 5055 patients, for 2053 BU patients, PCR tests were performed with 1746 (85.0%) positive PCR results. The other patients were diagnosed by clinical evaluation and did not receive an alternative diagnosis during the treatment. The trend of the treated BU patients of all four hospitals can be observed in figure 2. The number of patients steeply decreased between 2008 and 2013, from 897 patients to 492 patients respectively. Between 2013 and 2019 the decrease was less steep and reached 240 patients in 2019. Laboratory testing by PCR started in 2010, and increased from 23.1% to 54.2% in 2019. The confirmation rate (based on PCR IS2404) increased from 84.8 % in 2010 to 90.8% in 2019.

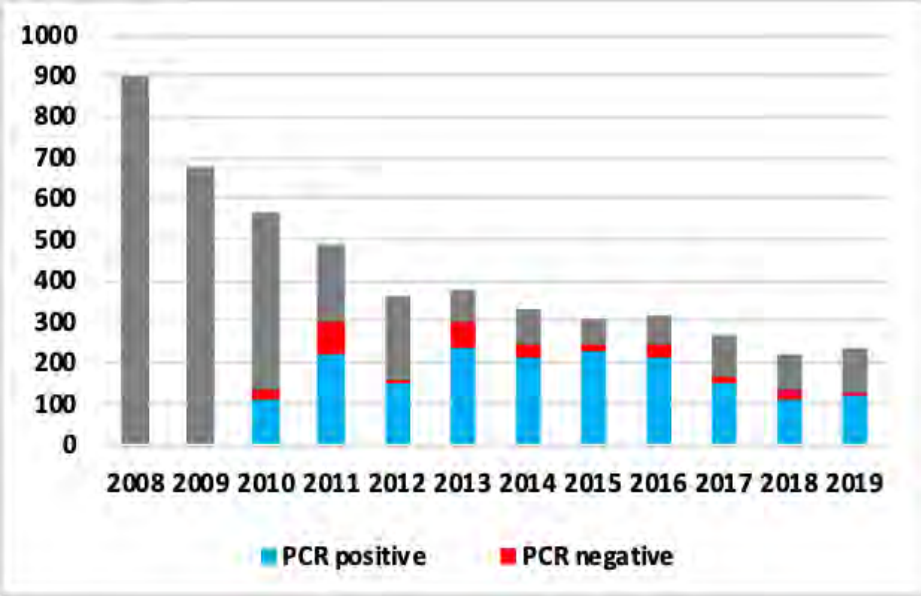

Figure 2: Number of BU patients in Benin, 2008-2019

Geography

The geographic distribution of treated BU patients (clinically diagnosed) reveals the endemic areas in southern Benin over time (figure 3). The endemic areas are all located around rivers and streams. The maps show a decrease in the number of BU patients between 2008, 2013, 2019. The relative geographical distribution over the different districts nevertheless has remained stable. In 2019, the highest endemic districts were Bonou, Adjohoun, and Dangbo.

The geographic distribution for BU patients who had their diagnosis confirmed by PCR was similar in all diagnostic and treatment centres in Benin.

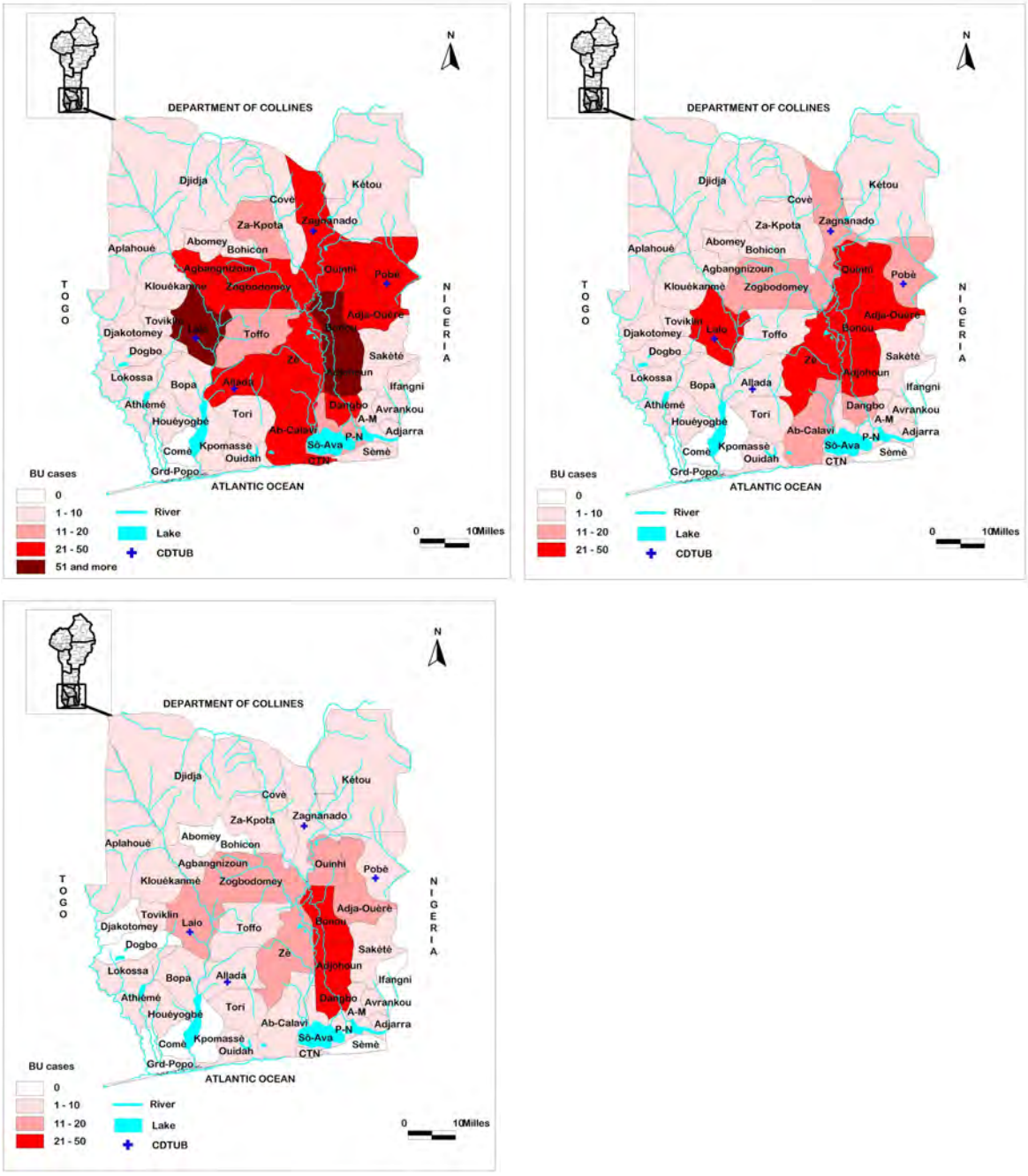

Figure 3 Map of epidemiology of BU patients in 2008, 2013, and 2019 respectively.

Clinical features per hospital

Out of the 5055 reported patients, 2600 were males (51.5%). The median age was 17 (IQR 9-36) years. One third of the BU patients reported in Zagnanado hospital. Almost half of the patients treated for BU during the study period had severe lesions (47.4%) with a predominance of ulcerative lesions (66.3%). A functional limitation at the start of treatment was observed in 25.4% of patients. Lesions were predominantly located on the lower limbs (62.8 %). The majority of the patients (93.8%) received antibiotic treatment. The characteristics of the BU patients per hospital are shown in table 1.

Table 1: The characteristics of the BU patients per hospital

	Allada n=766 (15.1%)	**Lalo n=989 (19.6 %)**	**Pobè n=1597 (31.6%)**	**Zagnanado n=1703 (33.7%)**	**Total n=5055**
Sex - male (%)	398 (52.0)	497 (50.3)	768 (48.1)	937 (55.1)	2600 (51.5)
Age in years - median (IQR)	14 (8-33)	14 (8-35)	14 (8-30)	25 (12-45)	17 (9-36)
severity – WHO category (total n=4999) *					
I (%)	219 (29.6)	234 (24.0)	318 (20.1)	35 (2.1)	806 (16.1)
II (%)	328 (44.4)	418 (42.9)	631 (39.8)	444 (26.1)	1821 (36.4)
III (%)	192 (26.0)	323 (33.1)	636 (40.1)	1221 (71.8)	2372 (47.4)
Ulcerative lesion *					
n = 5027 (%)	593 (11.8)	750 (14.9)	1150 (22.9)	841 (16.7)	3334 (66.3)
Body site*					
upper limb (%)	248 (32.5)	256 (26.0)	509 (32.1)	342 (20.1)	1355 (26.9)
lower limb (%)	435 (56.9)	578 (58.7)	927 (58.4)	1224 (72.0)	3164 (62.8)
upper and lower limb (%)	6 (0.8)	0 (0.0)	22 (1.4)	9 (0.5)	37 (0.7)
Other (%)**	75 (9.8)	151 (15.3)	129 (8.1)	124 (7.3)	479 (9.5)
Functional limitation*					
n = 4841 (%)	218 (4.5)	87 (1.8)	800 (16.5)	125 (2.6)	1230 (25.4)
PCR Performed***					
n = 3484 (%)	323 (67.9)	227 (41.1)	1156 (94.8)	347 (28.1)	2053 (58.9)
PCR IS2404 - positive					
n = 2053 (%)	260 (80.5)	172 (75.8)	1089 (94.2)	225 (64.8)	1746 (85.0)
Antimicrobial treatment *					
n = 4610 (%)	616 (81.8)	929 (95.6)	1179 (99.7)	1598 (93.8)	4322 (93.8)

* p<0.001, Pearson chi-square

** Other consists of head, face, thorax, abdomen, genital area, bone.

*** Data from 2010-2019 since laboratory confirmation by PCR was implemented from 2010 onward

Age pattern

Proportion of BU disease in children has decreased from 2008 to 2019. Less than 40% of the patients are children in the last three years. This trend is similar to the PCR confirmed BU patients (figure 4).

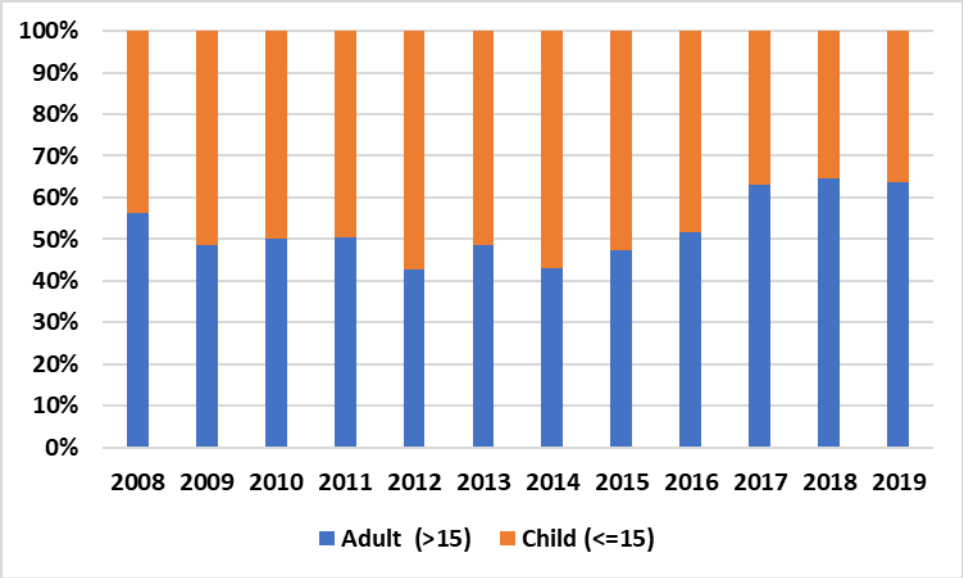

Figure 4: age distribution among BU patients

Severity of disease per hospital over time

Severe lesions of BU increased from 43.6% in 2008 to 60% in 2019. This increasing trend was observed at all the treatment centres, except for Pobè where the category three lesions went down from 44.9% in 2008 to 31.9% in 2019 (Figure 5).

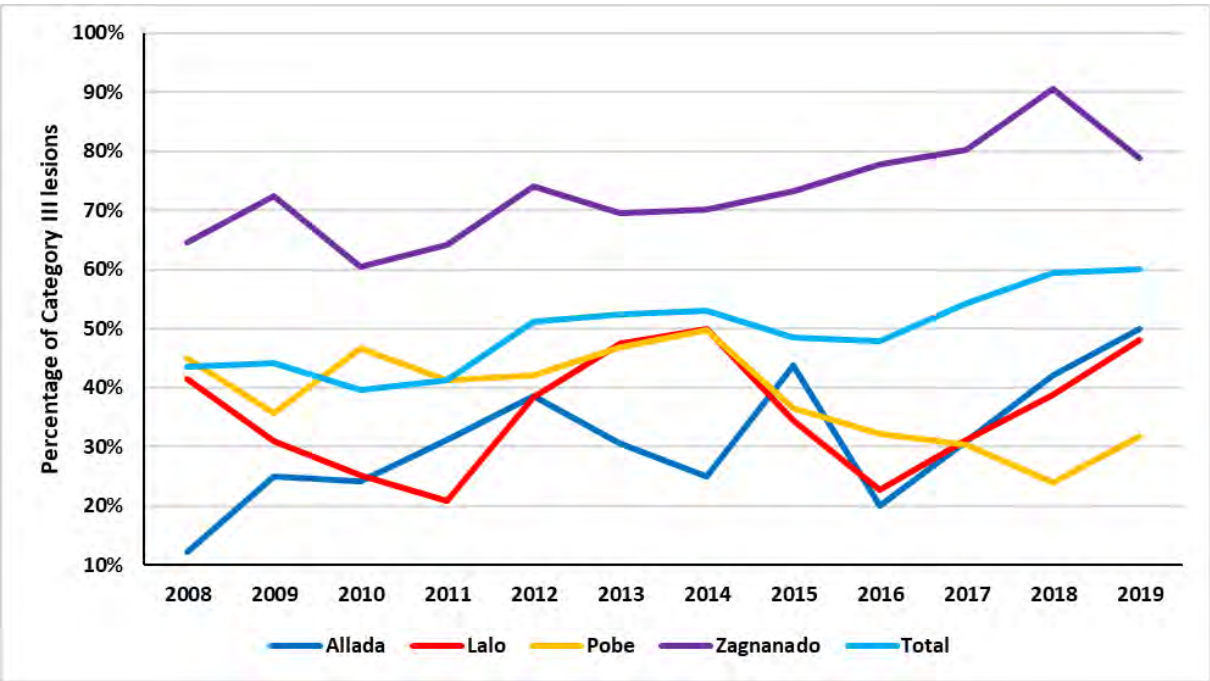

Figure 5: Disease severity (WHO Category III lesions) per hospital over the time

Discussion

We report on the epidemiology and disease severity of Buruli ulcer from 2008 to 2019 in Benin. A decrease was observed in almost a quarter in the number of Buruli ulcer patients - from 897 patients in 2008, to 240 patients in 2019. This trend has been observed in all the

endemic districts over the last decade. Nonetheless, this decrease in the number of BU patients has also been observed in other endemics countries in West Africa such as Ghana, Cameroon, and Côte d'Ivoire (14, 15, 31, 32). Currently, the system consists of community-based surveillance teams that includes village volunteers, teachers and community workers supervised by health workers (19, 20, 22, 23). A form developed by the WHO (WHO BU02) is used as the data collection tool for surveillance purposes (23, 24).

A reporting system like the one based on BU02 forms has its limitations; emerging foci and outbreaks of Buruli ulcer might be missed (33), and new foci of infection in remote areas might emerge. Due to the focused distribution of Buruli ulcer, not all clinicians are familiar with the diagnosis and patients might go undetected due to lack of training of medical personnel, or lack of access to health care. However, the national program in Benin typically responds to reports of ulcerative lesions from locales where BU has not been previously reported. Team of experts would move to areas where the suspected cases are reported. As an example, in August 2019, 17 patients were reported with suspected BU in Tchaourou, in the north of the country. The team of experts including two doctors from two BU referral hospitals, representatives of the national and regional health system, and an interpreter attempted to obtain diagnostic confirmation by PCR; none of the patients appeared to have BU (34). Indeed, we believe that large outbreaks of BU would not go unnoticed for a longer period of time in Benin, and we believe that the decline in incidence is real. Community activities have been conducted in endemic districts (19, 20, 22, 23), potentially resulting in a reduction in BU incidence. *M. ulcerans* is an environmental bacterium (35-37); various hygiene and sanitation programs may have impacted on BU incidence. Drilling of new water wells in Benin may have decreased the incidence of Buruli ulcer (38). Humans affected by discharging BU lesions may shed *M. ulcerans* into the environment (39, 40). The introduction of antibiotic therapy in the treatment of Buruli ulcer could also lead to a reduction in the mycobacterial load discharged into the environment, resulting in fewer new infections. Curiously, in some other endemic countries like Australia, Nigeria and Liberia, an increase in the number of new Buruli ulcer patients has been reported (7-9).

Despite this decrease in the number of patients observed in recent years, the proportion of category III lesions has increased over time - from 43.6% in 2008, to 60% in 2019. This increase in the disease severity has also been observed in Australia (12). The increase in lesion severity may be due to an increase in the pathogenicity of the mycobacterium through a genetic modification, a change in the structure of mycolactone, or an increase in the production of mycolactone inducing a more severe clinical manifestation (31) but it goes without saying that an alternative explanation could be a longer patients' or doctors' delay (41).

In this study, the median age was 17 years. This is similar to the median age in Togo with an age range of 1–72 years (42), a median age of 20 years (IQR 10-43) reported in patients from Ghana (15), and a median age of 20 years (IQR 13.5–42.5) in patients reported from

Nigeria (43). In a previous Buruli ulcer progress report on the period 2004–2008 in Benin (44), the median age was 12 years. Another study on the surveillance system for Buruli ulcer in Benin conducted from 2003 to 2006 reported a median age of 14 years (22); apparently, the median age of the affected population in Benin is increasing. Nevertheless, BU patients are still younger than those in Australia where median age was 54 years (range 1–95 years).

The WHO recommends microbiological confirmation of at least 70% of suspected Buruli ulcer cases in endemic countries (45). The reference test to date is PCR using the Insertion Segment 2404 (46-48). In Benin, confirmation by PCR is currently performed in two laboratories: the reference laboratory for mycobacteria in Cotonou and the laboratory of CDTUB Pobè set up by the Raoul Follereau Foundation. PCR testing increased from 23.1% to 54.2% of all BU suspects. Although only 54.2% of the suspected cases had a laboratory test, the PCR positivity rate was very high (from 84.8 % in 2010 to 90.8% in 2019). This mirrors the precision of the clinical diagnosis by experienced clinicians. A study on BU clinical diagnosis accuracy revealed that trained clinicians clinically diagnose BU with a sensitivity of 92% (95% CI, 85%–96%) and specificity of 91% (95% CI, 81%–96%) (49). However, the possibility of an overdiagnosis of Buruli ulcer remains. PCR requires training and laboratory skills, and it is expensive, and only available in specialized, centralized laboratories away from rural remote areas where Buruli ulcer is endemic (46). Establishing and maintaining the required quality of PCR is challenging, with a variable level of performance of the laboratories currently providing diagnostic services (50). New diagnostic tools for Buruli ulcer that can be used in peripheral health facilities are in dire need, and numerous studies have been carried out to develop a simple diagnostic test. A DNA amplification method called Loop Mediated Isothermal Amplification (also known as LAMP); and mycolactone detection by the fluorescent Thin-Layer Chromatography (fTLC); f-TLC may offer a new tool for confirmation of suspected Buruli ulcer cases (51-53). The WHO has recently proposed a clinical diagnosis score called "BURULI SCORE". It is a Multivariable Prediction Model for Diagnosis of *Mycobacterium ulcerans* Infection in Individuals with Ulcerative Skin Lesions. This score was found to have a good performance (54), but it still requires further evaluation of its effectiveness.

A potential limitation of our study is that we used reported data, based on the BU02 forms; we did not add field surveys like door-to-door assessments of any non-reported cases of Buruli ulcer, to assess possible under-reporting to the health system. Meanwhile, earlier over-reporting might also have played a role in the observed decline in incidence; in 2008, there were no PCR services in place for case confirmation yet. Another limitation is the completeness of the data. This is a retrospective study based on the use of the PNLLUB database. For some items, these were missing data – but as can be appreciated from the tables and figures, missing data were relatively few.
With this change in epidemiology which Benin, like other endemic countries, is facing, it is important to maintain the national surveillance system. Field surveys like the one we

described above, as well as random visits to sites would be a good approach to add to the passive case finding by the reporting system that is already in place.
Faced with the low and declining number of patients detected per year, optimal use of resources through the integration of surveillance of cutaneous NTDs and the training of health workers is necessary to maintain a viable health care system.

Acknowledgments: We thank all the staff from the four CDTUB, from the national control program and from the Ministry of health for sharing their patient's information. We express our sincere gratitude to Jean Gabin Houezo, coordinator of the PNLLUB in Cotonou.

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